

Neutrophil-mediated gastrointestinal injury

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Key Words: *Inflammation, Inflammatory bowel disease, Neutrophil, Nonsteroidal anti-inflammatory drugs*

Atteinte gastro-intestinale à médiation neutrophile

RÉSUMÉ : Les maladies inflammatoires du tractus digestif sont souvent caractérisées par une infiltration dense des neutrophiles dans la *lamina propria* et la migration trans-épithéliale subséquente de ces cellules vers la lumière du tube digestif. Si les neutrophiles jouent un rôle essentiel dans la défense contre l'infection bactérienne, ils peuvent également provoquer une atteinte significative dans les tissus hôtes. Les preuves quant au rôle des neutrophiles dans l'atteinte tissulaire significative propre à un certain nombre de troubles gastro-intestinaux et les mécanismes par lesquels les neutrophiles provoquent une atteinte tissulaire sont passés en revue ici. On aborde également les preuves à l'effet que certains anti-inflammatoires d'usage courant exercent des effets bienfaits par la modulation de l'extravasation ou de l'activation des neutrophiles.

The human immune system is a complex network of cells and humoral factors that are continuously interacting to maintain homeostasis within the body and prevent the invasion of foreign pathogens. Neutrophils (or polymorphonuclear leukocytes) account for 50% to 60% of the total circulating leukocytes (1) and act as a first line of defence against infectious agents or foreign substances that may penetrate the body's physical barriers. Neutrophils are capable of releasing an array of substances that can destroy cells and dissolve connective tissue. Unfortunately, neutrophils are intrinsically unable to differentiate between host and foreign antigen. As such, neutrophils are capable of mediating tissue-destructive events in inflammatory disorders, even in the absence of a pathogen. Thus, neutrophils have a paradoxical role in that they are essential for host defense, yet they are also involved in the pathogenesis of various inflammatory disorders.

The infiltration of a tissue by neutrophils is the hallmark of acute and chronic inflammatory disorders of the gastrointestinal tract (2). The recruitment and extravasation of leukocytes is essential to the inflammatory response. The *in vivo* requirements for cell trafficking are complex and involve multiple steps: circulation, adhesion, diapedesis and migration (Figure 1). Leukocytes in the circulation must overcome hemodynamic forces to adhere to the endothelial cell surface lining the vascular wall. As leukocytes exit capillaries, an outward radial movement towards the endothelial wall may be observed (1-3). This process, termed margination, is generally attributed to the fact that red blood cells overtake leukocytes in the axial flow of the greatly expanded diameter of the postcapillary venules. Following margination, the leukocytes may interact with the vascular endothelium via the coordinated expression of adhesion receptors on the surface of the leukocytes and counter-receptors on the

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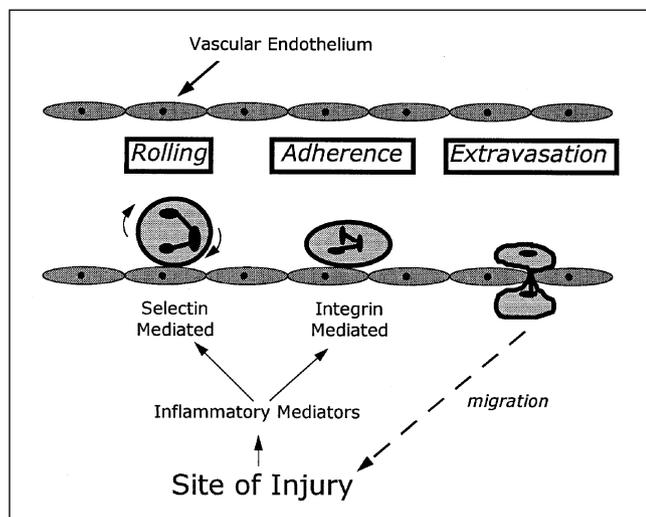


Figure 1) Schematic diagram illustrating the chain of events leading to neutrophil recruitment to a site of injury. The low affinity 'rolling' of neutrophils on the endothelium is mediated largely by selectins, while firm adherence of the neutrophil is mediated by binding of integrins to counter-receptors (members of the immunoglobulin super-family) on the endothelium. Once a neutrophil has extravasated, its migration towards the site of injury is regulated by chemoattractants released at the site of injury

endothelial surface. Some adhesion molecules are constitutively expressed, whereas others are only expressed after stimulation by a variety of substances such as interleukin-1beta (IL-1 β), lipopolysaccharide (LPS), tumour necrosis factor-alpha (TNF α), and interferon-gamma. The initial interaction between leukocyte and endothelium is of low affinity and results in the leukocytes 'rolling' along the endothelial surface. This interaction is subsequently strengthened, such that there is firm adhesion of the leukocyte to the endothelium, and the leukocyte becomes stationary. This adhesion is a prerequisite for the extravasation of these cells from the vascular endothelium into the adjacent interstitial cavity.

The adhesion molecules involved in leukocyte-endothelial interactions can be broadly divided into three main families: the selectins, the integrins and the immunoglobulin (Ig) super-family. The selectins are a family of adhesion molecules that mediate adhesive interactions among endothelium, leukocytes and platelets. Selectins are required for leukocyte rolling. L-selectin is constitutively expressed by most circulating leukocytes (neutrophils, monocytes, eosinophils) (2-4) and can bind to carbohydrate determinants found in several endothelial cell surface ligands, including P- and E-selectin (2-6). Upon activation, L-selectin is rapidly shed from the surface of neutrophils (4,7,8). C-reactive protein is capable of attenuating leukocyte adhesion to endothelial cells by accelerating the shedding of L-selectin from the neutrophil surface (9). P-selectin (CD62) is constitutively present on endothelial cells and platelets. In addition, P-selectin can be rapidly up-regulated on the endothelial and neutrophil cell surface by translocation of preformed molecules stored in Weibel-Palade bodies and alpha-granules, respectively (3). P-selectin expression

on platelets can initiate platelet-leukocyte interactions (10). E-selectin is restricted to endothelial cells, and its expression can be up-regulated in response to IL-1 β , TNF α and LPS (4,11,12).

The progression from rolling to firm adherence to the endothelium is dependent on leukocyte activation. Leukocyte integrins recognize Ig super-family ligands on the endothelium, such as intercellular adhesion molecule-1 (ICAM-1), intercellular adhesion molecule-2 (ICAM-2), and vascular cell adhesion molecule-1 (VCAM-1). Both ICAM-1 and ICAM-2 are constitutively expressed on endothelial cells. However, activation with cytokines, such as IL-1 β and TNF α , can up-regulate the expression of ICAM-1. Integrins are heterodimeric proteins consisting of associated alpha and beta subunits. The beta₂ integrins (CD11/CD18) are restricted to leukocytes and are the best characterized integrin family (3,13). These integrins consist of a common beta subunit (CD18) linked to one of three alpha subunits (CD11a, CD11b or CD11c). Like other members of the Ig super-family, integrin expression may be up-regulated by certain inflammatory mediators. CD11/CD18 integrins are basally expressed on leukocytes, but upon stimulation with inflammatory mediators, undergo a conformational change in order to support adhesion (13).

During inflammation, neutrophils that attach to the vascular endothelium may eventually migrate through inter-endothelial gap junctions into the underlying tissue. The transendothelial migration is mediated by platelet endothelial cell adhesion molecule-1 (PECAM-1) (14). Antibodies against PECAM-1 block neutrophil extravasation without affecting adherence to the endothelium (15). This adhesion molecule is concentrated mainly at the sites of cell-to-cell contact, with higher concentrations on the basal side of the cleft between adjacent endothelial cells (16).

Evidence implicating neutrophils as mediators of tissue injury is growing rapidly. A substantial amount of evidence has come from experiments involving monoclonal antibodies directed against leukocyte and endothelial cell adhesion molecules, and the observation that the reduction or attenuation of these interactions substantially reduces the amount of tissue injury. This review focuses on the role of neutrophils in several gastrointestinal diseases, the mechanisms by which neutrophils may produce tissue injury and therapeutic approaches to control neutrophil-dependent tissue destruction.

NONSTEROIDAL ANTI-INFLAMMATORY DRUG-INDUCED GASTROPATHY

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is severely limited by the ability of these drugs to cause gastric ulceration in a significant proportion of patients (17). The mechanism by which this occurs is still unknown, but is generally believed to be related to the ability of these agents to inhibit prostaglandin (PG) synthesis. It has also been proposed that leukocytes play a role in the pathogenesis of NSAID-induced gastropathy. The involvement of neutrophils in NSAID-induced injury is supported by observations

that the severity of gastric mucosal lesions induced in rats by administration of indomethacin can be significantly reduced by rendering the rats neutropenic before NSAID administration (18), and that treatment with monoclonal antibodies directed against the β_2 integrin on neutrophils (CD18) (19), ICAM-1 or P-selectin on the endothelial cells (20) can reduce leukocyte adhesion and the extent of injury induced by indomethacin. NSAIDs stimulate neutrophil adherence to postcapillary mesenteric venules in rats (21,22), possibly by increasing adhesion molecule expression. Andrews et al (23) reported that acetylsalicylic acid (ASA) and indomethacin augmented the expression of ICAM-1 by gastric blood vessels within 30 mins of administration, and that this could be prevented by pretreatment with a PG. Fiorucci et al (24) found that indomethacin was capable of inducing a time- and concentration-dependent up-regulation of CD11b and CD18 expression on neutrophils. Intravital microscopy studies of rat mesenteric venules have shown that superfusion with either indomethacin or ASA increased the number of adherent leukocytes and that this effect could be prevented by pretreatment with a lipoxygenase inhibitor, a leukotriene B_4 (LTB_4) antagonist, or PGs (21,22). These results support the concept that NSAIDs may create an imbalance between the pro- (LTB_4) and antiadhesive (PGI_2) factors present in the vasculature such that leukocytes become adherent. In addition, it has been shown that indomethacin administration results in increased plasma $TNF\alpha$ levels in both humans and animals (25-27) and that a significant correlation exists among the degree of gastric damage, neutrophil margination and $TNF\alpha$ release in rats treated with indomethacin (25,26). $TNF\alpha$ released in response to NSAID administration may account for the increase in ICAM-1 expression that has been observed within the gastric microcirculation (23) and, in turn, to the increased neutrophil adherence observed following NSAID administration (21,22).

While there is convincing evidence suggesting a primary role for neutrophils in NSAID-induced gastric injury, this is not necessarily the case for NSAID-induced small intestinal injury. Arndt et al (28) found that monoclonal antibodies directed against CD11b/CD18 and E-selectin were capable of reducing indomethacin-induced neutrophil infiltration into the intestine, but not the severity of jejunal ulceration. It was therefore postulated that granulocyte recruitment into the intestine occurred as a consequence, rather than a cause, of the mucosal injury. This conclusion is supported by the observations of Reuter et al (29) that luminal bacterial counts increased significantly in the small intestine following NSAID administration, but only subsequent to the first detection of mucosal injury.

HELICOBACTER PYLORI-ASSOCIATED MUCOSAL INJURY

Colonization of the human stomach with the Gram-negative bacterium *Helicobacter pylori* induces chronic active gastritis that is characterized by a dense mucosal infiltration of neutrophils (30,31). The severity of mucosal injury appears to be directly correlated with the extent of neutrophil

infiltration (32,33). *H pylori* colonizes the human gastric epithelium, residing primarily in the mucus layer adjacent to the epithelium (34). *H pylori* is capable of causing mucosal damage in at least two ways: by releasing substances that directly injure epithelial cells (35), and/or by releasing substances that induce the recruitment and activation of leukocytes. The persistence of the neutrophil response in chronic *H pylori* infection suggests that these cells are crucial to the inflammatory response and the resultant mucosal damage (36).

H pylori is a rich source of factors that are capable of recruiting and/or activating neutrophils. Similar to other Gram-negative bacteria, *H pylori* can liberate LPS (endotoxin) that is able to prime neutrophils for activation (37). Extracts of *H pylori* have been shown to be chemotactic for neutrophils (32) and capable of stimulating degranulation (38). All strains of *H pylori* appear to possess a gene (*napA*), albeit with variations in the level of expression, that encodes a 150 kDa neutrophil-activating protein. Water extracts of *H pylori* that contain this neutrophil-activating protein were found to contain another factor that increased the surface expression of CD11b/CD18 on neutrophils and increased their adherence to endothelial cells (30,31,39). In addition to up-regulating the expression of CD11b/CD18, the water extract of *H pylori* can prevent the shedding of L-selectin from the neutrophil surface (30). The continued presence of the L-selectin on the neutrophil surface was suggested to allow for continued neutrophil rolling along the endothelium so as to increase the probability of adhesion after stimulation with *H pylori* (30). It has previously been shown that *H pylori*-induced neutrophil adhesion to endothelial cells is CD11b/CD18- and ICAM-1-dependent (39), consistent with in vivo studies that have shown ICAM-1 up-regulation in patients with *H pylori*-associated gastritis (40). *H pylori*-infected mice exhibit a marked increase in the flux of rolling leukocytes and the appearance of platelet-leukocyte aggregates during infection (41). Furthermore, platelet P-selectin expression was enhanced in the *H pylori*-infected mice. Enhanced platelet P-selectin expression has also been found in *H pylori*-infected patients, as have circulating platelet aggregates, that are not observed in *H pylori*-negative patients (41). Activated platelets, expressing P-selectin, may adhere to neutrophils. Nagata et al (10) reported that the adherence of activated platelets to neutrophils through P-selectin results in an increased release of superoxide anion. Thus, the generation of oxidants may contribute to *H pylori*-induced tissue damage.

Interleukin-8 (IL-8) is a chemokine that is a potent chemoattractant and activator of neutrophils (42). *H pylori* is capable of inducing gastric epithelial cell lines to increase IL-8 mRNA expression and to secrete IL-8 (31,36). Crabtree et al (43) noted that this in vitro epithelial cell response is observed specifically with strains that carry the *cagA* gene, but that *cagA* is not the direct inducer of these chemokine responses. Rather, multiple gene products from the *cag* pathogenicity island are required for the induction of IL-8 (36,44-46). Of interest is the observation that infection with

cagA-positive strains is associated with greater inflammation scores (46,47), prominent neutrophil infiltration (48) and increased induction of the respiratory burst in neutrophils (49).

INFLAMMATORY BOWEL DISEASE

A prominent feature of chronic inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC) is a marked neutrophilic infiltrate in the intestinal lamina propria (50-52). Both clinical and experimental data exist implicating neutrophils in the pathogenesis of IBD. Studies in experimental models of colitis have revealed that monoclonal antibodies directed against the leukocyte integrin CD18 are capable of significantly reducing neutrophil infiltration into the colonic tissue and of attenuating the extent of colonic injury in both rabbits (51) and rats (52). Neutropenia was found to attenuate weight loss, colonic neutrophil infiltration and mucosal necrosis associated with dextran sulphate sodium-induced colonic damage in rats (53). Recently, an inhibitor of neutrophil activation that has been reported to induce shedding of L-selectin (54) and to interfere with functional expression of CD11/CD18 (55) was shown to reduce significantly the severity of experimental colitis in the rat (56).

Biopsies of actively inflamed mucosa from patients with IBD exhibit increased expression of beta₂ integrins, ICAM-1 and E-selectin (57). Koizumi et al (57) reported that neutrophils that had migrated into crypt abscesses stained positively for E-selectin. Another immunohistochemical study confirmed increased E-selectin expression in active IBD (58). In addition, these authors demonstrated enhanced staining of venules in inflamed mucosa for P-selectin and ICAM-1, while infiltrating neutrophils in similar areas of IBD activity exhibited enhanced integrin expression (CD11b/CD18). Furthermore, human intestinal mucosal microvascular endothelial cells derived from the mucosa of IBD patients exhibited markedly greater leukocyte binding capacity than those from normal mucosal samples (59).

Hommes et al (60) provided evidence for increased neutrophil infiltration and activation in IBD. Fc gamma receptor III (FcγRIIIb) is a low affinity neutrophil surface membrane IgG receptor. Soluble forms of the receptor (sFcγRIIIb) are released upon neutrophil activation, and elevated levels have been found at sites of inflammation (60). In gut lavages from both CD and UC patients, sFcγRIIIb concentrations were significantly increased. In addition, they found significantly elevated concentrations of LTB₄, a potent neutrophil chemoattractant and activator, and there was a significant correlation between sFcγRIIIb and LTB₄ lavage concentrations.

A prominent histological feature of UC is the crypt abscess, which is characterized by the presence of neutrophils within the lumen of crypts. After emigration of neutrophils into the lamina propria, they migrate across the epithelium and are excreted in feces. Interestingly, measurement of fecal neutrophil excretion (using a biochemical marker) has recently been suggested to be a sensitive, noninvasive marker

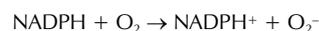
of disease activity in UC (61). Emigration of neutrophils across the epithelium can increase epithelial permeability (62) and stimulate epithelial secretion, at least in *in vitro* models (63). Thus, the transepithelial migration of neutrophils could contribute to the chronicity of inflammation by increasing the access of bacterial products to the lamina propria, as well as contributing to the generation of diarrhea.

MECHANISMS OF NEUTROPHIL-MEDIATED TISSUE INJURY

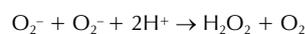
The same mechanisms that are required for the microbicidal actions of neutrophils are responsible for the tissue injury caused by these cells. Neutrophils use a combination of oxidative and enzymatic processes that act in a concerted manner. Upon activation of neutrophils, two events occur almost simultaneously: the oxidative burst (so termed because of the 50- to 100-fold increase in oxygen consumption that occurs) (1), and degranulation, which is the process of primary (azurophilic) and secondary (specific) granules fusing with the plasma membrane and discharging their contents into the extracellular medium and/or into a phagocytic vacuole. The oxidative burst results in the production of reactive oxygen metabolites (ROM) and possibly the production of reactive nitrogen metabolites (RNM). Neutrophils are also capable of releasing some cytokines, most notably IL1 and TNFα, which may contribute to the generation of tissue injury in some conditions (64). However, there is little, if any, direct evidence for a role for neutrophil-derived cytokines in promoting tissue injury other than through the promotion of further neutrophil recruitment into a tissue.

ROMs

The NADPH oxidase system is a latent plasma membrane-associated enzyme complex. Upon activation by agents such as LTB₄, platelet-activating factor (PAF), immune complexes, complement components or bacterial products, the enzyme system is rapidly activated resulting in the generation of large amounts of superoxide anion:



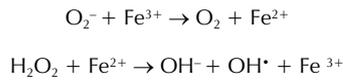
Superoxide anion may also be released from monocytes after binding to activated platelets (10) and endothelial cells. This occurs through the enzyme xanthine oxidase (65). Superoxide anion is generally unreactive towards most biological substrates (66,67). It serves, however, as a precursor for the production of more potent ROM. Hydrogen peroxide is rapidly formed by spontaneous dismutation and/or the catalytic action of superoxide dismutase (SOD):



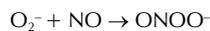
Hydrogen peroxide is a more powerful oxidant than superoxide anion, but is generally not reactive towards biological substrates unless present in high concentrations (ie, greater than physiological levels) (68). However, hydrogen peroxide has been found to possess some biologically relevant properties *in vitro*: it is capable of promoting unidirectional electrolyte transport (chloride ion secretion) in the rat ileum and

jejunum without any overt cytotoxic effects, possibly contributing to the diarrhea associated with IBD (68,69); it has been shown to increase mucosal permeability (68); and it has been demonstrated to be a potent mutagen (70).

A more important role for hydrogen peroxide may be in the formation of the highly reactive hydroxyl radical via the superoxide-driven Fenton reaction:



Some speculation exists as to the ability of neutrophils to produce hydroxyl radicals *in vitro*, but superoxide anion has been reported to be capable of releasing redox active iron from the iron storage protein ferritin, thereby providing the catalyst for hydroxyl radical formation (68,70). An alternative pathway for hydroxyl radical formation *in vivo* has been proposed. Beckman et al (71) suggested that nitric oxide, produced by neutrophils and phagocytes at sites of inflammation, may react with the superoxide anion to produce peroxynitrite anion:



The peroxynitrite anion is relatively stable under alkaline conditions. At physiological pH, however, protonation and subsequent formation of peroxynitrous acid may occur. Peroxynitrous acid is very unstable and rapidly decomposes.

The hydroxyl radical is an extremely reactive and destructive oxidant. It is very short lived and reacts with virtually all biomolecules at diffusion-limited rates of reaction (66). It is also conceivable that endothelial cell membranes may be oxidized to activate phospholipase A₂ leading to the production of potent neutrophil chemoattractants such as LTB₄ and PAF (72). Hydroxyl radicals are also capable of reacting with and depolymerizing gastrointestinal mucin, peroxidizing lipids, oxidizing proteins and promoting DNA strand scission (1,68,70,73). Nitrogen dioxide is also a very reactive free radical and may react with alkanes, alkenes, hemoprotein, and oxidize thiols and thioethers (66). Nitrogen dioxide may also initiate lipid peroxidation (66).

Neutrophils contain large amounts of the hemoprotein myeloperoxidase (MPO) (up to 5% of the dry weight of the cell) (50). Upon activation, this protein is secreted into the extracellular medium where it exerts little, if any, effect on its own (50). However, in combination with hydrogen peroxide, MPO catalyses the oxidation of halides (chloride, bromide, iodide) to their corresponding hypohalous acids (1,50,70). Because the plasma concentration of chloride ion is more than 1000 times the concentration of the other halides, hypochlorous acid is probably produced at most sites *in vivo* (50). Hypochlorous acid is extremely toxic. It possesses the two oxidizing equivalents of hydrogen peroxide and is approximately 100 to 1000 times more toxic than either superoxide anion or hydrogen peroxide (68,70). Due to the high reactivity of hypochlorous acid it does not accumulate within biological systems; rather, it rapidly reacts with avail-

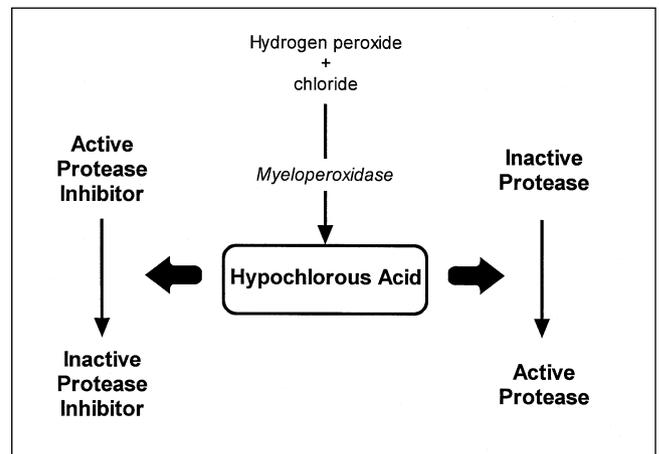
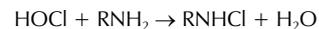


Figure 2) Hypochlorous acid generated by the neutrophil-derived enzyme myeloperoxidase can activate proteases and inactivate anti-proteases, thus favouring the digestion of connective tissue

able substrates such as sulfhydryls, polyunsaturated fatty acids, DNA, pyridine nucleotides, aliphatic and aromatic amino acids, and nitrogen-containing compounds (68). In addition, hypochlorous acid participates in the generation of chloramines (RNHCl). In this rapid and spontaneous reaction, hypochlorous acid reacts with primary amines (RNH₂) to produce derivatives containing the nitrogen-chlorine bond (50,68):



In general, chloramines are less toxic than hypochlorous acid, but they do retain the two oxidizing equivalents of hydrogen peroxide and hypochlorous acid (50,68,70).

MPO-derived hypochlorous acid and RNHCl can damage cells directly via sulfhydryl oxidation, hemoprotein bleaching, protein and amino acid degradation and inactivation of metabolic cofactors (NADPH) and DNA (50,68). These MPO-derived oxidants may also act to degrade and depolymerize components of the extracellular matrix (50,68). While superoxide anion is believed to be associated with hypochlorous acid only in its role as a precursor of hydrogen peroxide, there is evidence to suggest that superoxide anion may oxidize physiological substrates (eg, ascorbate) and thereby double the amount of hydrogen peroxide available to the MPO system (50).

ROM, and possibly RNM, may play a role in the activation of nuclear factor κB (NF- κB), an inducible transcription factor (70,74,75). NF- κB , in an inactive form, is found in the cytoplasm of cells, complexed to an inhibitor, I κB . Activation of NF- κB triggers the release of NF- κB from I κB , resulting in the translocation of NF- κB from the cytoplasm to the nucleus where it binds to DNA and regulates the transcription of specific genes. Most genes known to be activated by NF- κB are involved in immune and inflammatory reactions (eg, IL-1 β , IL-6, IL-8, interferon-beta and TNF α) (70,74). NF- κB genes also regulate the transcription of adhesion molecules involved in leukocyte-endothelial cell interactions such as E-selectin, ICAM-1 and VCAM-1

(70,75). Production of ROM and RNM may therefore perpetuate the inflammatory response by activating NF- κ B and increasing the expression of proinflammatory cytokines and adhesion molecules.

PROTEOLYTIC ENZYMES

Neutrophil granules contain over 20 enzymes (50), but those that have received the most attention are the serine proteinases (elastase and cathepsin G) and the metalloproteinases (collagenase and gelatinase). Elastase is an extremely potent enzyme that is capable of degrading almost all components of the extracellular matrix and is able to cleave a variety of key plasma proteins (eg, Igs, complement proteins and clotting factors) (50). Collagenase can cleave each of the interstitial collagens (ie, type I, II and III collagens) at a specific locus, while gelatinase degrades type V, XI and possibly IV collagens (50). Extracellular elastase activity is regulated by the presence of antiproteinases in plasma and interstitial fluids: alpha-proteinase inhibitor, alpha₂-macroglobulin and secretory leukoproteinase inhibitor. Alpha₁-proteinase inhibitor (formerly called alpha₁-antitrypsin) is the primary defence against uncontrolled elastase-mediated damage by irreversibly forming an enzyme-inhibitor complex at a rate that approaches the diffusion-controlled limit of elastase release (50). However, the presence of neutrophil-derived oxidants may alter this protease-antiprotease balance (Figure 2).

Chlorinated oxidants are capable of inhibiting anti-proteinases, such as alpha₁-proteinase inhibitor and alpha₂-macroglobulin (50). Simultaneously, they are also responsible for the activation of latent collagenase and gelatinase secreted by neutrophils (50), thereby creating a favourable situation for the degradation of the interstitial matrix and epithelial cells. Neutrophil elastase is capable of cleaving oxidized alpha₁-proteinase inhibitor. More important, activated neutrophils also release and express a metalloproteinase activity that directly inactivates native alpha₁-proteinase inhibitor; the activity of this metalloproteinase is also directly linked to hypochlorous acid (50). Thus, neutrophils use hypochlorous acid to inactivate alpha₁-antiproteinase by two independent processes. Once neutrophil elastase is tissue bound, even alpha₁-proteinase inhibitor cannot extricate or completely inactivate the enzyme (50). In the lung, the release of neutrophil elastase from neutrophils is capable of inducing IL-8 secretion by the bronchial epithelium, which in turn may recruit additional neutrophils (76). Treatment with recombinant secretory leukoprotease inhibitor attenuated both IL-8 levels and neutrophil numbers (77). It has also been found that neutrophil elastase expression could be up-regulated by inflammatory mediators such as TNF α and PAF (76). In the context of inflammation, it appears that neutrophils are capable of producing a self-perpetuating cycle to promote activation, recruitment and injury.

NEUTROPHILS AS THERAPEUTIC TARGETS

Several drugs currently used to treat inflammatory diseases in the gastrointestinal tract may produce their beneficial ef-

fects in part through modulation of neutrophil activation and/or adherence. In addition, the neutrophil may be a rational target for future therapeutic agents.

SULPHASALAZINE AND 5-ASA

Sulphasalazine is widely used in the treatment of mild to moderate active UC and colonic CD as well as in the maintenance of remission of UC (14). Oral sulphasalazine remains unmodified until it reaches the distal ileum and colon where it is metabolized by endogenous bacteria to yield 5-aminosalicylic acid (5-ASA) and sulphapyridine (78). It is well recognized that 5-ASA is the pharmacologically active moiety of sulphasalazine. 5-ASA has been shown to exert a wide array of pharmacological actions, but several of these, such as inhibition of LT and PG synthesis, only occur at concentrations well above those that are achieved in the colonic mucosa with the usual doses of this drug. On the other hand, 5-ASA is a potent antioxidant and free radical scavenger (78) capable of scavenging a variety of free radicals including the superoxide anion, the hydroxyl radical and the peroxy free radical. Recent work from Sandoval et al (79) has shown that 5-ASA is capable of attenuating peroxynitrite-induced cell injury and apoptosis in human intestinal epithelial cells. 5-ASA is also capable of chelating iron, thereby preventing formation of the hydroxyl radical and the degradation of deoxyribose (78). Moreover, 5-ASA can decompose neutrophil MPO, scavenge hypochlorous acid and selectively protect alpha₁-proteinase inhibitor against inactivation by hypochlorous acid (78).

NITRIC OXIDE AND NITRIC OXIDE-NSAIDS

Nitric oxide is a potent inhibitor of leukocyte adherence to the vascular endothelium (80). Inhibition of nitric oxide synthesis was found to result in extensive leukocyte adherence in the mesenteric microcirculation of the cat (81). It has also been found that nitric oxide is capable of inhibiting leukocyte adherence in rat mesenteric microcirculation by inactivating the superoxide anion (80). In this manner, nitric oxide may also prevent the generation of ROM and RNM. Nitric oxide appears to be capable of reducing adhesion molecule expression (82). For example, Gauthier et al (83) demonstrated that nitric oxide could inhibit the expression of P-selectin on endothelial cells. The ability of nitric oxide to prevent leukocyte adherence has been exploited in the development of novel NSAID derivatives that are complexed to a nitric oxide releasing moiety (84). These 'nitric oxide-NSAIDs' have been shown to inhibit leukocyte adherence to the vascular endothelium and to spare gastrointestinal blood flow (84). Moreover, the nitric oxide-NSAIDs have greatly reduced ulcerogenic actions in the stomach and intestine, while maintaining the anti-inflammatory, analgesic and antipyretic activities of the parent drugs (84-87).

CORTICOSTEROIDS

Corticosteroids are the mainstay of medical treatment of moderate to severe IBD. While the efficacy of these drugs relates to the multiplicity of targets in the inflammatory cas-

cade at which they act, recent evidence suggests that inhibition of leukocyte adherence may be one of the most important anti-inflammatory mechanisms. For example, corticosteroids inhibit the increase in neutrophil-endothelial adhesion caused by exposure to endotoxin, principally by inhibiting the endothelial cell expression of E-selectin and ICAM-1 (88). In addition, corticosteroids can inhibit the release of several inflammatory mediators that are known to influence leukocyte adherence to the vascular endothelium, such as TNF α , IL-1 β and PAF (89).

PGs

PGs such as prostacyclin and PG E₂ are very potent inhibitors of neutrophil activation and adherence (90-92). The observation that administration of a number of different NSAIDs leads to significant neutrophil adherence (21,22) suggests that PGs act as endogenous inhibitors of neutrophil adherence. It is not yet clear whether PGs directly modulate the expression of specific adhesion molecules, although Andrews et al (23) demonstrated a rapid up-regulation of ICAM-1 in the gastric microcirculation of the rat after indomethacin or ASA administration. Expression of CD11a/CD18 also increased following administration of an NSAID, although this expression lagged behind that of ICAM-1 (23). Inhibition of neutrophil adherence by PGs may contribute to the cytoprotective effects of these mediators in the gastrointestinal tract (93).

METHOTREXATE

Methotrexate has been employed in the treatment of IBD and a number of other inflammatory diseases. Its beneficial effects may be produced, at least in part, through modulation of adhesion molecule expression or function. Methotrexate has been shown to interfere with adherence of leukocytes to the endothelium (94-97). It has also been shown to prevent the changes in neutrophil rolling, adherence and emigration induced by PAF. Evidence suggests that adenosine mediates the effects of methotrexate on leukocyte adherence. Cronstein et al (95) demonstrated that methotrexate markedly elevated adenosine release from connective tissue cells. The inhibition of PAF-induced leukocyte adherence by methotrexate appeared to be adenosine dependent because adenosine deaminase reversed the observed effects (96). Because adenosine also impairs oxygen radical generation by neutrophils (and oxyradicals enhance leukocyte-endothelial adherence), it has been suggested that this 'indirect' mechanism accounts for the blockade of leukocyte adhesion and emigration that occurs with adenosine and methotrexate (96).

COLCHICINE

Colchicine is one of the longest used anti-inflammatory drugs, but like the corticosteroids, its mechanism of action has never been clearly delineated. It is now clear that at least some of the anti-inflammatory activity of this drug is attributable to modulation of adhesion molecule expression. Colchicine can inhibit leukocyte rolling along the

vascular endothelium (98). This appears to be due to a suppression of L-selectin expression on the leukocyte (99). Colchicine has also been shown to inhibit ICAM-1 expression and the ability of E-selectin to mediate leukocyte rolling (99).

ANTIBODIES AND ANTISENSE

Numerous experimental studies in animal models have used antibodies directed against adhesion molecules or inflammatory mediators and have proven their value in attenuating inflammation. For example, antibodies directed against CD18 (51,52) and an antibody against VCAM-1 (100) were shown to reduce the severity of colitis in rodents and cotton-top tamarins, respectively. Thus far, the use of antibodies against adhesion molecules in humans has not been successful. Prolonged blockade of these molecules may render the patient much more prone to infection, and the cost of antiadhesion molecule therapy may prove prohibitive. Moreover, there is a significant chance that the patient will develop antibodies directed against the antiadhesion molecule antibody, even when the latter has been 'humanized'.

An alternative approach to blocking adhesion molecule activity in humans is the use of antisense oligonucleotides. Recently, Yacyszyn and colleagues (101) reported preliminary data suggesting that an antisense oligonucleotide directed against ICAM-1 may have some utility in treating some patients with IBD.

ANTIOXIDANTS AND PROTEASE INHIBITORS

Many animal studies have used the administration of antioxidants to prevent leukocyte-endothelial interactions with very good results. Antioxidants, however, are not suitable for chronic use; SOD and catalase have circulating half-lives of only eight and 20 s, respectively (68). In addition, it has been shown that administration of large amounts of certain antioxidants, such as SOD, may actually exacerbate tissue injury and inflammation (68). On the other hand, in cystic fibrosis there is a profound infiltration of neutrophils into the lung associated with respiratory derangements (76,77). These neutrophils express an elastase on their surface that is able to induce epithelial damage directly and to act as a chemoattractant by promoting secretion of IL-8 (76). McElvaney et al (77) reported that a recombinant secretory leukoprotease inhibitor was capable of significantly reducing both the levels of IL-8 and the number of infiltrating neutrophils. More recently, Yamaguchi et al (102) reported that a neutrophil elastase inhibitor could reduce neutrophil chemoattractant production in the liver after ischemia-reperfusion – a disorder believed to be neutrophil dependent (2,72,73).

CONCLUSIONS

Neutrophils are highly destructive cells that are essential for host defense, but also contribute to various inflammatory diseases. This review has highlighted the role of neutrophils in several gastrointestinal diseases and the mechanisms responsible for their tissue destructive activities. While several

anti-inflammatory drugs exert actions that may include suppression of neutrophil infiltration and/or activation, better understanding of the molecular mechanisms through which neutrophils produce tissue injury may provide clues to the development of more selective neutrophil inhibitors. Such drugs may prove useful in the treatment of inflammatory diseases of the gastrointestinal tract.

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